Title

Distinctive Mechanisms and Patterns of Exudative Versus Tractional Intraretinal Cystoid Spaces as Seen With Multimodal Imaging.

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Distinctive mechanisms and patterns of exudative versus tractional intraretinal cystoid spaces as seen with multimodal imaging.

Short title: Exudative and tractional intraretinal cystoid spaces.

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Introduction:

Macular edema is defined as the abnormal accumulation of fluid within or below the neuroretina which may be associated with numerous retinal conditions, potentially resulting in significant vision loss.[1] Fluid accumulation causes the formation of cystoid spaces within the neuroretina in the macular region, as seen with optical coherence tomography (OCT). Although this entity has been extensively and thoroughly investigated in multiple clinical, histopathologic and laboratory studies, its pathophysiology and the mechanisms leading to decreased visual acuity are not yet fully understood.[1,2]

Remarkable advancements in retinal imaging technology and a rapid pace of innovation have recently led to an explosive interest in the study of many retinal disorders including macular edema. Newer imaging modalities such as OCT angiography (OCT-A) have provided a different perspective for the study of this condition.[2]

"*Multimodal imaging*" is becoming increasingly popular as a clinical and research resource and refers to the study and diagnosis of retinal diseases using different imaging modalities, each optimally displaying specific morphological and/or functional features.[3] By combining data from various imaging tools and taking advantage of the strengths of each technique, it is possible to gain unique insights into the mechanisms of retinal disease. In the specific case of macular edema, traditional dye-based techniques such as fluorescein angiography (FA) may be especially powerful in the evaluation of retinal vascular function, while non-invasive modalities such as cross-sectional and OCT-A are more effective in the analysis of retinal morphology.

Although macular edema may be classified according to a diverse spectrum of causative etiologies, it is often associated with a breakdown of the inner and/or outer blood retinal barrier and the subsequent formation of "exudative" intraretinal cystoid spaces. Differently, tractional macular disorders are often associated with "non-exudative" intraretinal cystoid spaces without vascular leakage.[4,5]

Traditionally, exudative intraretinal cystoid spaces have received the most attention from clinicians and investigators, as they are frequently encountered in common conditions such as diabetic retinopathy and age-related macular degeneration (AMD). The many intricate mechanisms leading to vascular leakage have been discussed in the literature.[1] In contrast to exudative cystoid spaces, the tractional subtype is poorly described.[5] As a result, the distinction between tractional and exudative intraretinal cystoid spaces is nebulous and no clear-cut criteria have been proposed to differentiate these conditions.

The differential evaluation of exudative versus tractional intraretinal cystoid spaces may be particularly challenging with the decline of classic dye-based imaging modalities in favor of recent non-invasive techniques such as OCT-A. This changing paradigm may have significant implications in daily clinical practice, as the management of

exudative and tractional intraretinal cystoid spaces may require different therapeutic strategies. However, since the report by Johnson published more than a decade ago,[5] minimal advancement has been made in the characterization of tractional intraretinal cystoid spaces using advanced retinal imaging.

Recent reports have illustrated novel insights into the morphology and biomechanics of the macular region, with a distinctive focus on Müller cells, a cellular subtype considered to be a key player in the development of both exudative and tractional cystoid spaces.[6,7] Such a pathoanatomical approach coupled with multimodal imaging analysis may help to distinguish exudative and tractional forms of intraretinal cystoid spaces, providing a better understanding of the pathophysiology of these conditions. Therefore, we designed this retrospective study incorporating recent biomechanical theories and multimodal imaging with the aim to better characterize the different forms of macular edema and determine clear-cut distinctions between tractional and exudative intraretinal cystoid spaces using advanced retinal imaging.

Methods:

A retrospective, observational chart review of consecutive patients diagnosed with intraretinal cystoid spaces in association with exudative and tractional macular disorders and seen by 6 retina specialists (A.G., D.S., J.P.H., R.T., A.C. and M.R.) at the Fatebenefratelli-Oftalmico Hospital (Milan, Italy), the Stein Eye Institute, University of California Los Angeles (Los Angeles, USA), the Lariboisière Hospital (Paris, France) and the Humanitas-Gavezzeni Hospital, Humanitas University (Bergamo, Italy) was carried out in agreement with the tenets of the Declaration of Helsinki. Institutional Review Boards of all European participating centers approved the study protocol and the retrospective collection or data and patient records. The University of California Los Angeles Office of Human Research Protection approved the study protocol with the IRB#16-000574.

Electronic and paper records of all patients diagnosed with intraretinal cystoid spaces in association with exudative and tractional macular disorders and evaluated at the participating centers between January 1, 2015 and June 30, 2019 were reviewed and analyzed. In all participating European centers cases were identified using administrative lists and institutional patient databases, while at the Stein Eye institute cases were identified using imaging databases or by a medical billing record search, using the International Statistical Classification of Diseases and Related Health Problems, Ninth Revision (ICD-9) diagnosis code 362.56 for macular pucker, 360.21 for progressive (high) myopia, and code 362.53 for cystoid macular degeneration.

Inclusion criteria were the presence of exudative and tractional macular disorders in association with intraretinal cystoid spaces as seen with OCT. Tractional macular disorders included epiretinal membrane (ERM), vitreomacular traction (VMT), idiopathic full-thickness macular hole, tractional lamellar macular hole and myopic

foveoschisis. Exudative macular disorders included AMD, diabetic retinopathy, central or branch retinal vein occlusion, Irvine-Gass syndrome and posterior uveitis.

The presence of dye leakage and/or pooling as seen with FA was considered a sign of exudative cystoid spaces.

Included cases required imaging with cross-sectional OCT, en-face OCT and OCT-A, blue light fundus autofluorescence (BFAF), and FA, performed in a single-day imaging session.

Exclusion criteria were incomplete or poor-quality retinal imaging, the presence of visually significant cataract or corneal opacities and history of intraocular surgery except for uncomplicated cataract surgery. Patients with microcystoid macular edema (MME), degenerative lamellar macular hole, end-stage AMD, Mac-Tel type 2, X-linked retinoschisis, retinitis pigmentosa, toxic and solar retinopathies were also excluded from the analysis. Patients with coexisting exudative and non-exudative cystoid spaces were also excluded from the analysis.

In all patients, cross-sectional OCT, BFAF and FA imaging were obtained with the Spectralis HRA-OCT (Heidelberg Engineering GmbH, Heidelberg, Germany) and reviewed with the Heidelberg Eye Explorer (version 1.8.6.0) using the HRA/Spectralis Viewing Module (version 5.8.3.0). Optos FA (Optos, Inc., Marlborough, MA, USA) was also performed in certain patients.

FA images were required to include the late recirculation phases, 10 minutes or longer after dye injection.

Spectralis OCT scan patterns were used for all measurements and all eyes displayed a volume scan of 20 x 15 degrees in scan area with 19 B-scans spaced 242µm apart. A single high-definition horizontal line at 30 degrees was also available in all cases. Some cases displayed alternatively a high-density 15 x 10 degrees volume scan, with 97 B-scans spaced 30µm apart.

In all patients, en-face OCT and OCT-A imaging were performed either with the RTVue XR Avanti AngioVue OCT-A Version 2017.1.0.151 (Optovue, Inc., Fremont, CA, USA) or the DRI Swept Source OCT Triton (TopCon Corp, Tokyo, Japan). In all cases, patients were imaged with a 3 x 3mm OCT-A macular raster scan area. All cross-sectional OCT, OCTA, FA and BFAF images were qualitatively and quantitatively reviewed by at least 2 independent retina specialists (A.G., M.R., J.P.H., D.S., I.C., A.A.).

Qualitative assessment included the evaluation of the following characteristics: presence of leakage, staining or pooling with FA, anomalous hyper and/or hypofluorescence patterns with BFAF, assessment of the shape, distribution and localization of hyporeflective intraretinal cystoid spaces as noted with cross sectional and en-face OCT, the presence of cone bouquet abnormalities as defined by previous literature,[7] and the presence of ellipsoid zone and/or external limiting membrane disruption. In addition, abnormalities of both the superficial capillary plexus (SCP) and deep capillary plexus (DCP) were assessed with OCT-A.

Quantitative assessment included the analysis of central foveal thickness (CFT), automatically measured by the Spectralis platform. Further, automated segmentation of all retinal layers in the early treatment diabetic retinopathy study (ETDRS) subfields was performed to analyze and compare the thickness of the inner nuclear layer (INL)

versus the outer nuclear layer plus Henle Fiber layer (ONL, HFL) in exudative versus tractional intraretinal cystoid spaces subtypes. Manual adjustments of the retinal layer boundaries were performed in case of inaccuracies of the automated Heidelberg proprietary software.

The area and number of intraretinal cystoid spaces were assessed with Image J software v1.52A (National Institute of Health, USA, http://imagej.nih.gov/ij/). Briefly, en-face 3x3 OCT scans segmented at the DCP and at the HFL-ONL were thresholded with Otsu's algorithm, then binarized and analyzed with the "measure" and "analyzed particles" automated function of the Image J software, as illustrated in Figure 1. Mean, minimum and maximum number of the cystoid spaces, and the mean, minimum and maximum area of the cystoid spaces were recorded.

Image J software was also used to quantitatively assess the vessel density at the DCP and SCP of the included eyes. Due to the low reproducibility of OCT-A scans between different platforms,[8] such analysis was carried out using only images taken with a single device (TopCon Swept Source DRI OCT), which was used to examine most of the included patients. Vessel density measurements were defined as a proportion of the 3 x 3 mm whole macula area. For the vessel density measurement, a binarized image with intensity thresholding with Otsu's method was chosen.

Visual acuity (BCVA) was recorded at each visit and reported in Snellen fraction, which was converted into logarithm of the minimal angle of resolution (LogMAR) values for statistical analysis.

Descriptive statistics were calculated for all variables of interest. Mean and standard deviation values were calculated for continuous variables, while frequency and percentage were calculated for categorical variables. Analysis of variance test (ANOVA) was used to compare the statistically significant difference in continuous measurements among all subgroups. Univariate linear regression was used to investigate the differences among tractional and exudative groups for continuous covariates and logistic regression was used for categorical covariates. All analyses were conducted using Stata 16.0 software (StataCorp, college Station, TX, USA).

Results:

In total, 72 eyes from 69 patients, of which 37 were female and 32 were male, with a mean age of 70 ± 12 years (range 28-94 years) met the inclusion criteria and were enrolled in the study.

Multimodal imaging analysis illustrated specific morphologic and functional characteristics of exudative and tractional intraretinal cystoid spaces.

Exudative intraretinal cystoid spaces (Figure 2) were diagnosed in 36 out of 72 eyes (50%) and included the following etiologies: Irvine-Gass syndrome (12 out of 36 eyes, 33.3%), diabetic macular edema (7 out of 36 eyes, 19.4%), central/branch retinal vein occlusion (6 out of 36 eyes, 16.7%), infectious retinitis (2 out of 36 eyes, 5.6%), age-related macular degeneration (3 out of 36 eyes, 8.3%), and ERM (6 out of 35 eyes, 16.7%).

Exudative cystoid spaces were of roundish morphology, hyporeflective as seen with OCT, and located mainly in the INL, HFL and ONL. Cystoid spaces could coalesce to occupy all these layers, as

noted with cross sectional OCT (Figure 3 A and B).

Cross sectional and en-face OCT signs of traction such as retinal wrinkling and retinal folds were rarely present in this subtype of cystoid spaces and were only noted in cases of ERM in 6 out of 36 eyes (16.7%). Abnormalities of the central cone bouquet were identified in 18 out of 36 eyes (50%).

En-Face OCT displayed a characteristic “petaloid” morphology, noted in both the INL and ONL-HFL segmented images, with multiple smaller cystoid spaces located in the INL and bigger spaces located within the ONL-HFL.

This petaloid morphology was also identified with FA imaging with late-phase dye leakage and pooling of fluorescein into the cystoid spaces.

In all exudative cases, BFAF imaging showed well-defined round areas of hyperautofluorescence in the macular area, co-localizing with the hyporeflective spaces noted with cross sectional and en-face OCT and with the hyperfluorescent spaces with FA.

Tractional intraretinal cystoid spaces (Figure 4) were diagnosed in 24 out of 72 eyes (33.3%). Etiologies included tractional lamellar macular hole (12 out of 24 eyes, 50%), ERM (7 out of 24 eyes, 29.2%), myopic foveoschisis (2 out of 24 eyes, 8.3%), VMT (2 out of 24 eyes, 8.3%), and toxoplasmic retinochoroiditis (1 out of 24 eyes. 4.2%). In contrast to the exudative subgroup, signs of retinal traction were identified in all eyes (24 out of 24, 100%, $p<0.001$).

Tractional cystoid spaces were mainly located in the HFL, although non-confluent, roundish INL cystoid spaces were occasionally noted with cross sectional OCT. Unlike the exudative subtype, INL cystoid spaces did not coalesce with those located in the HFL (Figure 3, C). Further, in most of the cases (23 out of 24 eyes, 96%) cystoid spaces did not expand below the HFL-ONL boundary as in the exudative counterpart (Figure 3, C).

En-Face OCT segmented at the ONL-HFL displayed a characteristic radial “spoke wheel” pattern centered on the fovea. Of note, there was no evidence of leakage with FA, even in the very late phases.

Similar to exudative cystoid spaces, BFAF imaging illustrated hyperautofluorescence patterns in the central fovea, but without any specific morphology.

Eyes with idiopathic full-thickness macular hole constituted a third subgroup and 12 out of 72 eyes (16.7%, Figure 5) displayed this etiology. This group showed cross-sectional OCT, OCT-A and BFAF features of both exudative and tractional cystoid spaces, but without any leakage or pooling with FA. Such eyes displayed a characteristic “sunflower” appearance as seen with en-face OCT. As in exudative cystoid spaces, multiple INL and HFL-ONL cystoid spaces were present.

In full thickness macular hole, BFAF illustrated a central well-defined hyperautofluorescent circle, corresponding to the RPE exposed by the hole.

Multimodal features of exudative, tractional and full-thickness macular hole associated intraretinal cystoid spaces are summarized in Table 1, whether their en-face OCT patterns are illustrated in Figure 6.

Mean visual acuity was significantly different between the three subgroups ($p<0.001$). Eyes with tractional cystoid spaces exhibited significantly better visual acuity (0.19 ± 0.14 LogMAR, 20/30 Snellen

Equivalent) compared to the group with exudative macular edema (0.4 ± 0.14 LogMAR, 20/50 Snellen Equivalent, p=0.001) and full-thickness macular hole (0.84 ± 0.24 LogMAR, 20/140 Snellen Equivalent, p<0.001).

The analysis of dichotomous variables revealed significantly higher prevalence of INL cystoid spaces, cone bouquet abnormalities, EZ and ELM disruption in exudative versus tractional cystoid spaces (p=0.007, p=0.007, p<0.001 and p<0.001, respectively).

CFT was similar between eyes with exudative cystoid spaces and those diagnosed with the tractional subtype (p=0.251) and those diagnosed with full-thickness macular hole (p=0.465), without significant differences between the three subgroups (p=0.440).

INL thickness in the central and inner ETDRS subfields was significantly lower in eyes with tractional cystoid spaces versus the exudative and full thickness macular hole subgroups (p<0.001). Contrastingly, in the outer ETDRS subfield there were no differences in INL thickness between the three subtypes (p=0.316).

In all ETDRS subfields, differences in HFL-ONL thickness among the three subgroups were significant only in the central ETDRS subfield, with thicker HFL-ONL in the exudative subtype (p=0.020). Quantitative cross-sectional OCT retinal thickness analysis is summarized in table 2.

There were a greater number of INL cystoid spaces in both the exudative and full-thickness macular hole groups versus the tractional group (p=0.001). However, no significant differences were encountered in the number of HFL-ONL cystoid spaces between the three subgroups (p=0.107).

Differences in mean area of INL cystoid spaces were significant among the three subgroups (p<0.001), with greater values encountered in eyes with exudative cystoid spaces and full-thickness macular hole. On the other hand, no significant differences were encountered in the mean area of ONL cystoid spaces between the three subgroups (p=0.064).

Qualitatively, the SCP and DCP appeared to be anatomically preserved in eyes with both tractional and full-thickness macular hole-associated cystoid spaces. By contrast, a significant disruption of both the SCP and DCP was encountered in eyes with exudative cystoid spaces.

Quantitative analysis of the SCP and DCP showed a remarkable reduction of the microvascular density in eyes with exudative cystoid spaces, with significant differences if compared to both tractional and full-thickness macular hole subgroups (p=0.019 and p<0.001, respectively).

Quantitative en-face and OCT-A analysis are summarized in Table 3.

Discussion

Macular edema is one of the major causes of vision loss and may complicate various retinal diseases of different etiology.[1,4] It can be defined as an abnormal accumulation of intraretinal fluid, in which the normal fluid balance within the retina is disrupted such that fluid production exceeds fluid reabsorption.[2,9] Fluid leakage may accumulate in the retina mainly in extracellular cystoid spaces, presumably located

in the interstitial space.[2] Although the pathophysiology, morphology and content of intraretinal fluid have been thoroughly investigated with histology, FA and OCT over the years, the precise mechanisms leading to macular edema are not yet fully understood.[1,2,10-14]

Similar to the brain, the retina contains a fully functional hydrated interstitial space. Fluid normally flows in a vitreous to choroid direction due to osmotic gradient.[8,9,15-17] In physiological conditions the retinal interstitial space is relatively dry, as the volume of interstitial fluid is strictly controlled by the retinal capillary plexus and the retinal glial cells, mainly the Müller cells, which can regulate fluid absorption via aquaporin channels.[2] This delicate equilibrium can be altered by RPE and vascular dysfunction, the action of mechanical forces, or any combination thereof.

Cystoid macular edema due to retinal vascular disorders, i.e. “exudative”, can have various etiologies, but in most simplistic terms is the result of breakdown in either the inner (i.e. retinal vascular) or the outer (i.e. RPE) blood-retinal barriers. Abnormal capillary permeability leads to the extravasation of fluid from the vascular bed and its expansion in the interstitial space, which may result in tissue distortion and the formation of cystoid spaces within the retina (Figure 7 A, B). This phenomenon is partly compensated by mechanisms favoring fluid re-absorption, otherwise the cystoid spaces would expand indefinitely.[2]

Retinal capillaries are normally impermeable to proteins, electrolytes and water-soluble non-electrolytes. However, when there is a breakdown of the blood-retinal barriers, retinal edema can be understood in terms of the basic principles of capillary filtration, i.e. Starling's law.[9] Accordingly, all cases of exudative macular edema in this study illustrated a compromise of the blood-retinal barriers, confirmed by dye leakage or pooling with FA and by the presence of cystoid spaces in both the INL and HFL, contiguous with the deep retinal capillary plexus. Exudative cystoid spaces displayed a characteristic petaloid pattern noted with en-face OCT. The morphological characteristics of this subtype of cystoid spaces assessed with multimodal imaging were similar across different retinal conditions.

Intraretinal cystoid spaces may also be observed in tractional macular disorders, and their analysis illustrated a completely different pathway. This subgroup of eyes did not show any disruption of the blood-retinal barriers as supported by the absence of dye leakage and pooling and the near-normal thickness of the INL.

In tractional disorders, mechanical forces may physically separate and displace Müller cells processes in the HFL. Consequently, the interstitial fluid may flow freely within such anatomical “spaces”, created and maintained by traction and independent from Starling’s equation (Figure 7 C,D). In such a state, the intraretinal spaces may be physically maintained by the action of tractional forces and may not resolve unless such forces are released.

This phenomenon is normally referred to as retinal “schisis”, defined as the separation, or “splitting” of the neurosensory retina.[5,18,19] The typical cross-sectional OCT morphology of macular schisis (i.e. in tractional lamellar macular hole or in myopic foveoschisis) illustrates thicker retinas characterized by hyporeflective spaces intermingled by hyperreflective columnar elements.

A recent physical and mathematical model proposed that parafoveal *Z-shaped* Müller cells may increase retinal compliance and dampen the effect of mechanical forces due to the horizontal processes located in the Henle fiber layer, the preferred location of schisis in the retina.[6] Such horizontal processes may verticalize under the action of more severe tractional forces. Therefore, the intraretinal hyperreflective columnar elements illustrated with OCT may correspond to “beveled” or “verticalized” Henle fibers. Consistent with such a hypothesis, the “spoke wheel” en-face morphology noted in tractional cystoid spaces in this study relates to the anatomical configuration of the radially oriented Müller cell processes extending from the foveal center.[20]

The differences between exudative and tractional intraretinal cystoid spaces are not academic and may have a significant impact in current clinical practice.

The management of exudative cases necessitates mainly a medical approach with either intravitreal Anti-Vascular Endothelial Growth Factor (VEGF) drugs, intravitreal and/or systemic corticosteroids and/or topical non-steroidal anti-inflammatory agents.[1,2]

By contrast, a surgical strategy may be preferred in cases of tractional intraretinal cystoid spaces. The release of tractional forces may address the basic pathoanatomical abnormality, as traction creates and maintains “schitic” intraretinal spaces. Once the tractional component is removed, interstitial fluid may be reabsorbed by the RPE pump and the Müller Cells, with the slow restoration of the macular microstructure.[23-25]

We speculate that the content of the intraretinal cystoid spaces may differ in cases of exudative versus tractional etiology. In the exudative subgroup the blood-retinal barriers are compromised and the intraretinal fluid may assume the characteristic of an exudate. The recent OCT-A description of suspended scattered particles in motion, referring to the presence of lipid in Brownian motion within cystoid spaces, in various retinal vascular diseases may support this assumption.[26] On the other hand, tractional cystoid spaces may be comprised of transudative fluid. Different optical density in exudative versus degenerative cystoid spaces has already been described in the literature and is consistent with our hyphothesis.[27]

Whether the presence of tractional intraretinal cystoid spaces in the macular region should in effect be considered as “*macular edema*” it’s a matter of controversy, as this term classically refers to fluid accumulation due to vascular leakage. Although the term “*tractional cystoid macular edema*” has already been introduced by Johnson to describe the appearance of tractional cystoid spaces in vitreomacular traction syndrome, there is yet no consensus on such terminology.[4]

It should be noted that a component of exudation may occur in tractional disorders. In fact, in the present report a relevant proportion of ERM eyes displayed exudative patterns, suggesting that in some cases traction may lead to retinal vascular disruption with blood-retinal barrier breakdown. Further, vascular and tractional cystoid spaces are not mutually exclusive and may coexist in the same eye as suggested by the case illustrated in Figure 8. In cases in which the morphology of intraretinal cystoid spaces is not informative, FA imaging is seminal in the distinction between tractional and exudative subgroups.

BCVA was significantly higher in eyes with tractional versus

exudative intraretinal cystoid spaces. Relatively preserved BCVA in schitic conditions such as tractional lamellar macular hole has been previously described.[18] The preservation of good vision despite relevant anatomical alterations is difficult to explain. However, a recent report suggested a significant association between verticalized Henle fibers and lower BCVA, possibly due to an increase in the Müller cell's stiffness and consequent photoreceptor tractional damage.[6] Most of the eyes included in the present report were diagnosed with tractional lamellar macular hole, in which Henle fibers were mainly beveled, and not fully vertical. With such an anatomical configuration, Henle fiber may maintain a good reservoir of stiffness, which may protect the underlying photoreceptors.

Conversely, in exudative macular edema progressive fluid expansion may cause both acute, reversible vision loss (possibly due to altered light transmission to the photoreceptors) and chronic irreversible visual acuity decrease due to various factors including retinal ischemia, irreversible neural retinal damage and reactive glial alterations.[1,2,9] Accordingly, in the present report signs of photoreceptor damage such as ELM-EZ disruption were significantly higher in cases of exudative macular edema.

Intraretinal cystoid spaces associated with full-thickness macular hole displayed morphological and functional characteristics which were common to both tractional and exudative subgroups. In our study cohort, none of the included eyes diagnosed with full-thickness macular hole showed any signs of vascular exudation with FA. None of the patients diagnosed with full-thickness macular hole presented with other coexisting exudative or tractional macular pathologies.

While both antero-posterior and tangential traction play a major role in the pathogenesis of idiopathic full-thickness macular hole,[28] the morphology of such lesions with both cross-sectional and en-face OCT were different from typical tractional edema patterns and were more consistent with exudative cases.

Although the reasons of this discrepancy are unclear, it may be speculated that retinal hydration from the vitreous may play a role, as illustrated in Figure 9. Cystoid spaces in full-thickness hole are mainly located at the edge of the hole, in areas in which the neuroretina is partly detached from the RPE. Therefore, the capacity of the RPE pump to remove retinal fluid may be compromised in this region. Under these circumstances, transudative fluid entry may be favored over fluid reabsorption, with expansion of the cystoid spaces without frank vascular impairment. The enlargement of the cystoid spaces due to the inflow of fluid may produce an exudative-like morphology as seen with both cross-sectional and en-face OCT.

The "hydration" theory, already proposed by Tornambe, is consistent with the early observation by Sato et al., who postulated the existence of an inward-oriented passive fluid flow generated by a Na+-dependent inward current of viable photoreceptor outer segments.[17,29]

In chronic macular hole with flat edges such inward fluid flow may be defective, as suggested by the described lower prevalence of macular edema in chronic versus "acute" lesions.[30] A recent report showing resolution of full thickness macular hole edema with laser photocoagulation seems to support this assumption.[31]

The pathophysiology of intraretinal cystoid spaces in the macular region may involve other pathways distinct from exudation and

traction, including degenerative cellular loss and retrograde trans-synaptic degeneration, among others.[32-36] Cases of “degenerative” cystoid spaces were not included in our sample, as such analysis lies outside the aims and scope of this report and will be approached in future research.

This study has several limitations, including its retrospective design, which may have caused ascertainment bias. Further, the full spectrum of exudative and tractional macular diseases could not be exhaustively studied. Specifically, only few patients with myopic foveoschisis and vitreomacular traction were included. The proportion of tractional and exudative macular diseases included and analyzed in this study did not reflect that of the general population, a fact which could potentially cause selection bias. Although the imaging protocol was consistent between centers, two different OCT-A devices were used, a fact which may have limited the strength of our results. However, to reduce this bias, OCT-A sub-analysis was carried out using images from a single device.

Strengths of this report included a multimodal imaging approach in all cases, an adequate study sample size, robust quantitative and statistical analysis.

To conclude, the present study describes the morphological and functional characteristics of exudative and tractional intraretinal cystoid spaces in the macular region, which may have potentially relevant clinical implications. Exudative intraretinal cystoid spaces occurs in the presence of vascular impairment with disruption of the SCP and DCP. It is characterized by leakage and a characteristic petaloid pattern with FA, BFAF and en-face OCT. By contrast, tractional macular edema occurs without vascular impairment and with preserved DCP and SCP. It fails to display leakage with FA and it is characterized by a spoke-wheel morphology with en-face OCT. Further, signs of mechanical stress including radial retinal folds and/or wrinkling are always present. Intraretinal cystoid spaces associated with full-thickness macular hole displayed features common to both tractional and exudative subtypes, and retinal hydration may play a key role in its development.

A multimodal approach was seminal in the distinction between the different subtypes of intraretinal cystoid spaces and should be applied in normal clinical practice to increase the clinician’s diagnostic ability and improve patient’s clinical and surgical management. Despite the trending clinical application of noninvasive dye-free imaging modalities, traditional dye-based imaging such as FA maintain a key role in the distinction between cystoid spaces subtypes, particularly in cases where cross-sectional and en-face OCT may be inconclusive.

Larger prospective studies are required to confirm our findings, increase the external validity of this report and further explain the pathophysiology of exudative and tractional intraretinal cystoid spaces in the macular region.

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FIGURE LEGENDS

Figure 1. En-face Optical Coherence Tomography quantitative analysis.

A. En-face 3 mm x 3mm scan segmented at the Henle fiber layer-outer nuclear layer in an eye affected with Irvine-Gass syndrome, tractional lamellar macular hole and full-thickness macular hole. **B.** Automated thresholded image obtained with Image J software using Otsu's thresholding algorithm. **C.** Automated Image J software binarization of the image. Note that the central hyporeflective circular area correspondent to the full thickness macular hole was manually removed, as it does not represent a "*true*" cystoid space. **D.** Automated quantitative analysis with the "set scale" and "count particles" features of Image J software.

Figure 2. Exudative intraretinal cystoid spaces: multimodal features.

A. Cross sectional optical coherence tomography (OCT) scan of an eye affected with Irvine-Gass syndrome illustrates multiple hyporeflective cystoid spaces in both the inner nuclear layer (INL) and outer nuclear layer (ONL)-Henle fiber layer (HFL). A foveal detachment is present, as well as disruption of the Ellipsoid zone (EZ) and external limiting membrane (ELM). **B.** Fluorescein angiography illustrates dye leakage and pooling in a classic petaloid pattern. **C.** Blue fundus autofluorescence (BFAF) illustrates multiple areas of

hyperautofluorescence in a petaloid pattern, similar to FA. **D.** En-face OCT segmented at the level of the deep retinal plexus. Multiple small hyporeflective INL cystoid spaces are displayed in a swarm pattern around few larger and deeper cystoid spaces which extend into the ONL-HFL. **E.** En-Face OCT segmented at the level of the ONL. Larger HFL-ONL cystoid spaces are displayed in a petaloid pattern, similar to FA and BFAF. **F.** OCT-angiography (OCT-A) segmented at the level of the superficial plexus, which appears preserved. **G.** OCT-A segmented at the level of the deep retinal plexus shows disruption of the capillary matrix and hyporeflective flow voids corresponding to cystoid spaces in the INL.

Figure 3: Cross-sectional features of exudative and tractional intraretinal cystoid spaces.

A, B. Two eyes affected with Irvine-Gass syndrome. The hyporeflective cystoid spaces in the inner nuclear layer (INL) may merge (white star) with those located in the Henle fiber layer-outer nuclear layer (HFL-ONL). Different from cases of tractional macular edema, intraretinal cystoid spaces may expand over the boundaries of the HFL up to the external limiting membrane (black star). **C. Tractional intraretinal cystoid spaces.** In this patient affected with tractional lamellar macular hole, INL cystoid spaces do not merge with those located deeper in the retina. Further, intraretinal cystoid spaces do not extend past the HFL boundary.

Figure 4. Tractional intraretinal cystoid spaces: multimodal features.

A. Cross-sectional optical coherence tomography (OCT) scan of an eye affected with tractional lamellar macular hole. Multiple hyporeflective cystoid spaces are located in a schitic pattern in the HFL. A tractional epiretinal membrane is present. No outer retinal disruption is visible. **B.** Very late phase fluorescein angiography (FA) at 10 minutes illustrates no leakage and no pooling. **C.** Blue fundus autofluorescennce (BFAF) illustrate subtle hyperfluorescence in the foveal area, with a radial pattern. **D.** En-face OCT segmented at the level of the deep retinal plexus. Limited hyporeflective areas are visible centrally, corresponding to deeper intraretinal cystoid spaces located in the outer retina. **E.** En-face OCT segmented at the level of the ONL. Larger HFL cystoid spaces are displayed in a spoke-wheel radial pattern. **F, G.** OCT-angiography illustrates preserved deep and superficial capillary plexuses.

Figure 5. Full-thickness macular hole associated intraretinal cystoid spaces.

A. Cross-sectional Optical Coherence Tomography (OCT) scan of an eye affected with full-thickness macular hole. Multiple hyporeflective cystoid spaces are located in both the inner nuclear layer (INL) and outer nuclear layer (ONL). The edges of the hole are elevated, with neuroretinal separation from the retinal pigment epithelium (RPE). **B.**

Very late phase fluorescein angiography (FA) at 10 minutes illustrates no leakage and no pooling. A central circular area of hyperfluorescence is seen due to RPE exposure. **C.** Blue fundus autofluorescence (BFAF) illustrates a central area of hyperautofluorescence due to RPE exposure. **D.** En-face OCT segmented at the level of the deep retinal plexus. Multiple small hyporeflective INL cystoid spaces are displayed in a radial pattern around a large circular hyporeflective area correspondent to the edge of the hole. **E.** En-face OCT segmented at the level of the ONL. Larger ONL-HFL cystoid spaces are displayed in a “sunflower” pattern. The en-face morphology of this lesion exhibits features of both tractional and exudative macular edema. **F, G.** OCT-angiography illustrates preserved deep and superficial capillary plexuses.

Figure 6. En-Face distinctive patterns in tractional, exudative, and full-thickness macular hole-associated intraretinal cystoid spaces.

A. Exudative intraretinal cystoid spaces. Petaloid pattern. Multiple hyporeflective cystoid spaces display a petaloid pattern. **B. Tractional intraretinal cystoid spaces.** Spoke-wheel pattern. Multiple hyporeflective cystoid spaces radiating from the foveal center. **C. Full-thickness macular hole associated intraretinal cystoid spaces**. Sunflower pattern. Multiple hyporeflective cystoid spaces displayed in a radial pattern radiating from a central circular hyporeflective area.

Figure 7. Proposed pathophysiological differences between exudative and tractional intraretinal cystoid spaces.

A, B. Exudative intraretinal cystoid spaces. A. Vascular disruption leads to an increase in capillary permeability and fluid exudation following the rules of Starling’s equation (yellow arrows). **B.** According to Spaide, a gradient of fluid exists between the superficial and deep vascular plexus leading to fluid accumulation starting in the inner nuclear layer and expanding to the outer nuclear layer and Henle fiber layer.[2] Müller cells and the surrounding cellular architecture are displaced and distorted by the expansion of fluid into the retina. **C, D. Tractional intraretinal cystoid spaces. A.** No vascular disruption is present. **B.** The development of a contractile epiretinal membrane leads to the transmission of mechanical stress over the retina, with consequent Müller cell displacement. Traction creates spaces within the cells, which is filled with interstitial fluid flowing physiologically from the vitreous to the choroid (yellow arrows). Such spaces are created and maintained by traction, and do not resolve unless the traction is released. The movement of fluid does not follow the rules of Starling’s equation as there is no vascular impairment.

Figure 8. Coexistence of tractional and exudative intraretinal cystoid spaces in the same eye.

This patient was affected with both exudative age-related macular

degeneration and a tractional epiretinal membrane. The patient was excluded from the study due to the coexistence of tractional and exudative cystoid spaces. **A.** Fluorescein angiography (FA) illustrated dye leakage correspondent to the exudative hyporeflective cystoid spaces over the choroidal neovascularization. **B.** Cross-sectional optical coherence tomography (OCT) illustrated the simultaneous presence of both tractional (white star) and exudative (black star) macular edema. **C, D.** En-face OCT 3mm x 3mm and 6mm x 6 mm scans segmented at the outer nuclear layer and Henle fiber layer illustrate both tractional spoke wheel pattern (white star) and petaloid exudative pattern (black star). **E, F.** After intravitreal anti-vascular endothelial growth factor therapy, exudative macular edema resolved, as illustrated by the disappearance of petaloid cystoid spaces as see with en-face OCT and the absence of leakage or pooling as seen with FA imaging. As traction was not released, tractional macular edema persisted.

Figure 9. Proposed pathophysiological mechanism in full-thickness macular hole associated intraretinal cystoid spaces.

In full-thickness macular hole, the neuroretina at the edge of the hole is frequently detached from the underlying retinal pigment epithelium (RPE). According to Sato et al., an inward osmotic gradient may be present between the rod-cone layer and the internal limiting membrane favoring the flow of intersitial fluid through the retina.[17] Such an inward-directed gradient is physiologically compensated by the outward-directed pump of the retinal pigment RPE. When the neuroretina is detached, at the edge of the hole, the inward flow of fluid overcomes the outward-directed RPE pump, which is ineffective in removing all the fluid from the interstitium. This process results in fluid overload within the retina and the formation of cystoid spaces without any vascular disruption. As the osmotic gradient promote retinal hydration, the cystoid spaces may expand and compress the surrounding cellular components, similarly to exudative macular edema. Differently, in areas in which the contact between the neuroretina and the RPE is intact, the inward osmotic gradient is counterbalanced by the outward-directed RPE pump, and intraretinal fluid accumulation is averted.